Effects of Drinking Water Treatment on Susceptibility of Laying Hens to *Salmonella enteritidis* During Forced Molt

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ABSTRACT Feed deprivation is used in the layer industry to induce molting and stimulate multiple egg-laying cycles in laying hens. Unfortunately, the stress involved increases susceptibility to *Salmonella enteritidis* (SE), the risk of SE-positive eggs, and incidence of SE in internal organs. Leghorn hens over 50 wk of age were divided into 4 treatment groups of 12 hens each in experiment 1 and 3 treatment groups of 12 hens in experiments 2 and 3; hens were placed in individual laying hen cages. Treatment groups were 1) nonmolted (NM) and received feed and distilled water for 9 d, 2) force molted by feed removal for 9 d and received distilled water, 3) force molted by feed removal for 9 d and received 0.5% lactic acid (LA) in distilled water. An additional group (4) in experiment 1 only was force molted by feed removal for 9 d and received 0.5% acetic acid in distilled water. Seven days before feed removal hens were exposed to an 8L:16D photoperiod, which was continued throughout the experiment. Individual hens among all treatments were challenged orally with 10^4 SE on d 4 of feed removal. When compared with the NM treatments, weight losses were significantly higher in the M treatments, regardless of water treatments. When compared with NM treatments, crop pH was significantly higher in the M treatment receiving distilled water. Crop pH was reduced to that of the NM controls by 0.5% acetic acid in the drinking water. No consistent significant changes were observed for volatile fatty acids. The number of hens positive for SE in crop and ceca after culture and the number of SE per crop and per gram of cecal contents were higher in the M treatments, when compared with the NM treatments, but there was no effect of addition of either of the acids to the drinking water. Additional research using different acid treatment regimens may provide a tool for reducing the incidence of SE in eggs and internal organs during and following molting of laying hens.

(*Key words*: *Salmonella enteritidis*, molting, laying hen, lactic acid, acetic acid)

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INTRODUCTION

Salmonellosis is 1 of the most common foodborne diseases with an estimated 800,000 to 4 million human infections reported each year in the United States alone (Chalker and Blaser, 1988; Council for Agricultural Science and Technology, 1994; Angulo and Swerdlow, 1999). During the past 10 to 15 yr, the number of cases of gastroenteritis due to *Salmonella enterica* subspecies *enterica* serovar Enteritidis (SE) infections has increased markedly in the United States and Europe (Holt et al., 1995). By 1995, SE comprised 25% of all foodborne *Salmonella* isolates, compared with 5% in 1985 (Gomez et al., 1997). Epidemiological studies have attributed outbreaks of SE infections to the consumption of contaminated grade A table eggs (St. Louis et al., 1988). Between 1985 and 1991, 82% of SE infections in the United States were associated with table eggs (Mishu et al., 1994). From 1996 to 1999 SE illness rates declined 48%, and from 1996 to 2000 the incidence per 100,000 people decreased from 2.5 to 1.8 (US Department of Health and Human Services—Centers for Disease Control, 2000). Unfortunately based on the most recent epidemiological reports, this previously reported dramatic decline in SE incidence has now been eliminated by a renewed upsurge in SE infections (US Department of Health and Human Services—Centers for Disease Control, 2003). SE is invasive in poultry and, therefore, has the potential to contaminate eggs by transovarian transmission following colonization of the intestinal tract (Thiagarajan et al., 1994). Periodic clusters of contaminated

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Abbreviation Key: BGA = brilliant green agar; LA = lactic acid; M = molted hens; M-LA = molted hens receiving lactic acid; NA = nalidixic acid; NM = nonmolted hens; NO = novobiocin; TT = tetrathionate; VFA = volatile fatty acids.

eggs produced by laying hens may be related to stress incurred from specific management practices such as molting (Poppe, 1999).

Molting is a natural process in avian species that allows birds to renew their feathers in response to shorter days or cooler weather before annual migration and is not associated with the laying cycle (North and Bell, 1990). Because of high egg production capabilities, domesticated chickens do not experience complete molting until the end of an extensive laying period (North and Bell, 1990). However, as laying hens age the number of eggs produced decreases (Etches, 1990). If natural molting is allowed to proceed, nearly 4 mo are required for feathers to be lost and a new set grown (North and Bell, 1990). To add a second productive egg laying cycle in commercial laying operations, birds can be artificially induced to molt rapidly before the end of a laying cycle, rested, and returned to egg production within a span of 6 to 8 wk (North and Bell, 1990). Thus, a producer must decide whether to retire the flock and bring a new flock into production or recycle the current flock by induced molting to achieve a second lay cycle (North and Bell, 1990; Holt, 1999). This decision is based on the price of eggs vs. the cost of feed and replacement pullets (North and Bell, 1990). Generally, induced molting is economically advantageous, especially when factors are included such as the declining value of Leghorn hen carcasses and availability of fewer processors willing to process them (Webster et al., 1998; Holt, 1999). Compared with nonmolted birds, molted birds have increased productivity, increased egg quality, improved feed efficiency, and decreased mortality (Lee, 1982; Alodan and Mashaly, 1999). Molting therefore appears to have a beneficial effect on individual hen performance and an overall flock management advantage of having synchronized hens for a second laying cycle (Lee, 1982). The advantages of induced molting to producers are evident by the fact that 60% of the estimated 240 million laying hens nationwide and 90% in California are force molted, and the practice is growing more popular (Bell, 1987; Holt, 1999).

Feed withdrawal is the primary method used in the layer industry to induce molting and stimulate multiple egg-laying cycles in hens (Brake, 1993; Holt, 1995). The complete removal of feed for 10 to 14 d combined with a reduction in photoperiod from 16 to 8 h remains the method of choice (Bell, 1987; Brake, 1993) because the birds are out of production for a relatively short time (Brake, 1993). Flocks molted by feed removal require 9 to 10 wk before resuming optimum egg production, which is usually 80 to 90% of the maximal lay achieved during the initial cycle. Unfortunately, the stress associated with feed withdrawal causes increased susceptibility to SE infection, marked by increased intestinal shedding and dissemination of SE to internal organs such as the liver, spleen, and ovary (Holt and Porter, 1992; Holt, 1993; Thiagarajan et al., 1994; Holt et al., 1995). The incidence of SE-positive eggs increases 3-fold within the first 10 wk after hens are subjected to forced molting (USDA-Animal and Plant Health Inspection Service, 1994). Consequently, practices such as feed withdrawal, which increase the susceptibility of laying hens to SE infection, increase the risk of human salmonellosis from SE-contaminated eggs.

Methods to reduce SE colonization of laying hens force molted by feed removal are needed by the laying hen industry. Researchers at our laboratory (Corrier et al., 1997) have reported that addition of lactose to the drinking water of molting hens decreases colonization by SE. Further research at our laboratory indicates that this lactose method appears to be highly dependent on water consumption as well as other factors, making consistent results difficult to obtain.

The purpose of the research reported herein was to add lactic acid (LA) and acetic acid to the drinking water of Leghorn hens during feed deprivation and determine the subsequent effects on the environment of the gastrointestinal tract and susceptibility to SE.

MATERIALS AND METHODS

Bacteria

We used a primary poultry isolate of SE (phage type 13A) from the National Veterinary Services Laboratory (Ames, IA), selected for resistance to novobiocin (NO) and nalidixic acid (NA) at the USDA-Agricultural Research Service (Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, TX). Media to culture the resistant isolate in experimental studies contained 25 μg of NO and 20 μg of NA per milliliter. The challenge inoculum was prepared from an overnight culture that had been previously transferred 3 times in trypticase soy broth. The culture was serially diluted in sterile phosphate-buffered saline to a concentration of approximately 10^4 cfu/mL. The colony-forming units of the challenge inoculum were confirmed by plating onto brilliant green agar (BGA) plates.[3]

Molt Procedure

Feed deprivation by a modification (Holt, 1993) of a previously described procedure (Brake et al., 1982) was used to induce molt. Seven days before feed removal, hens were exposed to an 8L:16D photoperiod, which was continued throughout the experiment. Beginning on d 0, feed was withdrawn for 9 d, after which the studies were terminated.

Experimental Protocol

Single Comb White Leghorn hens[4] over 50 wk of age were obtained from a local commercial laying flock. Cloacal swab samples were collected from each hen and examined for salmonellae by successive culturing in tetrathionate (TT) broth BGA as described by Andrews et

[3]Difco Laboratories, Detroit, MI.
[4]Hyline International.

al. (1992). *Salmonella* spp.-positive hens were eliminated from the studies. Laying hens were placed in wire layer cages (1 hen per cage) and were provided free access to distilled water and a balanced unmedicated corn-soybean-meal based mash layer diet that met or exceeded National Research Council requirements for nutrients (1994). This diet provided 2,818 kcal of ME/kg, 16.5% CP, 3.5% calcium, and 0.48% available phosphorus. Before use, 3 randomly selected 25-g samples of the feed were cultured successively in buffered peptone water, TT broth, and BGA as described by Andrews et al. (1992) and examined for salmonellae. *Salmonella* spp.-positive feed was not found. Hens were allowed to acclimate for 1 wk followed by random assignment to 4 treatment groups of 12 hens each designated as either (1) nonmolted controls (NM); (2) feed deprived (M); (3) M hens given distilled water containing 0.5% LA (M-LA); or (4) M hens given distilled water containing 0.5% acetic acid in experiment 1. The 0.5% acetic acid treatment was not used in experiments 2 and 3.

On d 4 of molt for both molting procedures, all hens in all groups were challenged by crop gavage with 1 mL of inoculum containing approximately 10^4 cfu of NA-NO-resistant SE. The challenge dosage approximates the dosages of 5.6×10^4 cfu reported previously to be the mean infectious dosage (ID_{50}) for SE in nonmolted hens (Holt, 1993).

On d 9, the last day of the studies, all hens were euthanized, and the crop, ceca, liver, spleen, and ovaries were excised aseptically. Crop pH and LA concentrations were determined as described previously (Durant et al., 1999). Crop pH was determined by insertion of a sterile glass pH electrode[5] through an incision in the crop wall, ensuring that the electrode remained in contact with the crop mucosal surface (Durant et al., 1999). Each crop was excised and cut open aseptically, and the entire crop and contents together with 10 mL of sterile distilled water were blended[6] for 1 min. Samples of the blended crop were cultured and analyzed for concentrations of LA. The crop, ceca, liver, spleen, and ovary of each hen were cultured separately for SE, except in experiment 1 in which the liver and spleen were combined and cultured.

Crop Total Volatile Fatty Acids and LA Concentrations

The concentrations of volatile fatty acids (VFA) in the crop contents and cecal contents were determined by gas-liquid chromatography as described previously (Corrier et al., 1990). Briefly, analyses were conducted with a gas chromatograph equipped with a flame ionization detector and peak profiles integration-quantification integrator.[7] Each sample peak profile was integrated and quantified relative to an internal standard of methylbutyric acid placed in the same sample. Analyses were conducted at an oven temperature of 200°C and a flow rate of 85 mL/min. Lactic acid concentrations were determined by an enzymatic method (Hohorst, 1965).

Cecal VFA Concentrations

The concentrations of propionic acid and total VFA (acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids) in the cecal contents were determined by gas-liquid chromatography as described by Corrier et al. (1990) and briefly outlined above.

Crop Colonization by SE

One milliliter of the blended crop sample was transferred into 10 mL of TT broth and incubated for 24 h at 37°C. After incubation, the broth was streaked onto NO-NA-BGA plates, incubated for an additional 24 h at 37°C, and examined for the presence of suspect SE colonies. Suspect colonies were confirmed by biochemical tests on triple sugar-iron agar and lysine-iron agar[8] and further identified as SE serologically using *Salmonella* O antiserum group D, factors 1, 9, 12. Identification of the NO-NA-resistant SE by the culture on NO-NA-BGA plates and by the biochemical and serological procedures described were considered confirmatory without further serotyping.

Cecal Colonization by SE

One cecum from each hen was cut into several pieces, placed in 30 mL of TT broth, shaken vigorously, and incubated for 24 h at 37°C. After incubation, the broth was streaked on NO-NA-BGA plates, incubated for an additional 24 h at 37°C, and examined for the presence of suspect SE colonies. Suspect colonies were confirmed biochemically and serologically as described in the section on crop colonization.

SE Concentration in Cecal Contents

The contents of one cecum from each hen were serially diluted and spread plated on NO-NA-BGA plates at dilutions 10^{-1} through 10^{-4}. The plates were incubated for 24 h at 37°C, after which the number of colony-forming units of SE per gram of cecal contents were determined, and SE colonies were confirmed biochemically and serologically as described in the section on crop colonization. Cecal contents in which SE was not detected at the 10^{-1} dilution on BGA plates and after TT broth enrichment and BGA plating were scored as 0 cfu. Cecal contents negative at 10^{-1} dilution on BGA plates but positive after TT enrichment and BGA plating were arbitrarily assigned log 0.95 cfu of SE per gram of cecal contents.

[5]Model 05669-20, Cole Palmer, Niles, IL.
[6]Stomacher 80 Lab Blender, Stewart Medical, London.
[7]Model 110 Gas Chromatograph, SR1 Instruments, Torrance, CA.
[8]Oxoid, Unipath Ltd., Hampshire, UK.

TABLE 1. Effect of treatments on BW changes, crop pH and lactic acid (LA), cecal propionic acid (PA), and total volatile fatty acids (TVFA) in experiment 1

	Parameter[1]				
Treatment[2]	BW change from NM (%)	Crop pH	Crop LA (μmol/crop)	Cecal PA (μmol/g of cecal content)	Cecal TVFA (μmol/g of cecal content)
NM	0	4.89[b]	49[a]	37.2[a]	146.7[a]
M	–25	6.02[a]	21[ab]	25.0[b]	102.6[b]
M-LA	–26	4.93[b]	30[ab]	26.5[ab]	111.0[ab]
M-AA	–26	5.24[b]	16[b]	28.9[ab]	118.5[ab]
LSD[3]		0.59	32	11.5	40.9

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Values represent the mean of 12 hens per treatment.

[2]NM = full fed-nonmolted; M = feed deprived; M-LA = feed deprived + 0.5% lactic acid in distilled water; M-AA = feed deprived + 0.5% acetic acid in distilled water.

[3]LSD = least significant difference as determined by Fisher's protected LSD procedure.

Liver, Spleen, and Ovary Colonization by SE

Liver, spleen, and ovary specimens were minced with scissors and cultured according to NPIP guidelines (USDA—Animal and Plant Health Inspection Service, 1989). The organ samples were incubated for 24 h at 37°C in TT broth. After incubation, the broth was streaked onto NO-NA-BGA plates, incubated for an additional 24 h at 37°C, and examined for the presence of SE colonies. Suspect colonies were confirmed biochemically and serologically as described in the section on crop colonization.

Statistical Analysis

Chi-squared analysis was used to determine significant differences between treatment groups for incidences of SE colonization of the crop, ceca, liver, and spleen (Luginbuke and Schlotzhauer, 1987). Differences in the cecal pH, VFA, and LA concentrations, $\log_{10}$ *Lactobacillus* counts, and log colony-forming units of the SE counts among treatment groups were determined by ANOVA using GLM procedures. Significant differences were further separated using Fischer's protected least significant difference (LSD) procedure and commercial statistical analysis software (Luginbuke and Schlotzhauer, 1987). All data were analyzed by individual trial, and statistical analyses were considered significant at ($P < 0.05$).

RESULTS

In experiment 1 there was a decrease from the original BW of approximately 25% for hens deprived of feed for 9 d (Table 1). The acid treatments did not affect the reduction in BW observed for the hens deprived of feed. The pH of the crop was significantly higher in the M treatment when compared with the other groups. The only change in crop LA was a decrease in the molted hens given acetic acid when compared with the NM (full fed) hens. When compared with the NM hens, propionic acid and total VFA were reduced in the M hens that received no acid in the drinking water. The number of hens in experiment 1 with SE culture-positive crops, ceca, and livers and spleens was significantly increased in the M hens with or without the acids (Table 2). These values ranged from 0 to 1 per 12 hens in the NM hens to 6 to 12 per 12 hens in the M hens. The numbers of *Salmonella* in the crop and ceca were increased in the M hens with or without acids in the drinking water when compared with the NM hens.

TABLE 2. Effect of treatments on incidence and severity of *Salmonella* Enteritidis (SE) colonization and invasion in experiment 1

	Parameter[1]				
	SE culture-positive hens (n)			$\log_{10}$ *Salmonella*	
Treatment[2]	Crop	Ceca	Liver and spleen	Crop	Ceca
NM	1/1	1/12	1/12	0.22[c]	0[b]
M	7/12*	12/12*	10/12*	2.09[ab]	6.17[a]
M-LA	10/1	9/12*	12/12*	3.19[a]	4.76[a]
M-AA	6/12*	12/12*	9/12*	1.63[b]	5.08[a]
LSD[3]				1.32	2.48

[a–c]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Values represent the mean of 12 hens per treatment.

[2]NM = full fed-nonmolted; M = feed deprived; M-LA = feed deprived + 0.5% lactic acid in distilled water; M-AA = feed deprived + 0.5% acetic acid in distilled water.

[3]LSD = least significant difference as determined by Fisher's protected LSD procedure.

*Significantly ($P < 0.05$) different from NM control by chi-squared analysis.

TABLE 3. Effect of treatments on BW changes, crop pH and lactic acid (LA), cecal propionic acid (PA), and total volatile fatty acids (TVFA) in experiment 2

	Parameter[1]				
Treatment[2]	BW change (%)	Crop pH	Crop LA (μmol/crop)	Cecal PA (μmol/g of cecal content)	Cecal TVFA (μmol/g of cecal content)
NM	–3	4.61[b]	129[a]	31.4[a]	117.5[a]
M	–23	5.53[a]	16[b]	22.0[b]	95.4[ab]
M-LA	–28	4.97[b]	28[b]	16.2[b]	70.8[b]
LSD[3]		0.52	51.5	6.8	27.1

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Values represent the mean of 12 hens per treatment.

[2]NM = full fed-nonmolted; M = feed deprived; M-LA = feed deprived + 0.5% lactic acid in distilled water.

[3]LSD = least significant difference as determined by Fisher's protected LSD procedure.

In experiment 2, decreases in original BW of 3, 23, and 28% were observed for the NM, M, and M-LA treatment hens, respectively (Table 3). Crop pH was significantly higher for the M hens than for the other 2 treatments. Crop LA and cecal propionic acid were lower in the M and M-LA treatment hens, and total VFA were significantly lower in the M-LA hens. The number of hens in experiment 2 with SE culture-positive crops was higher in the M-LA hens when compared with the NM hens. The number of hens with culture-positive ceca was higher in the M and M-LA treatment hens; however, there was no difference in the number of hens with SE culture-positive liver and spleen (Table 4). When compared with the NM hens, the number of *Salmonella* in the crop was significantly higher in the M-LA treatment hens, and the number of *Salmonella* in the ceca of the M and M-LA treatment hens was significantly higher.

The effect of treatments on BW changes, ovary weights, crop pH, crop LA, and total VFA for experiment 3 are shown in Table 5. Reductions in BW from the original weights were 2, 23, and 25% for the NM, M and M-LA treatment hens, respectively. Ovary weights for the M and M-LA treatment hens were less than half the weight of the NM treatment hens. When compared with the NM treatment hens, crop pH was significantly higher in the M and M-LA treatment hens, whereas crop LA was significantly reduced. Cecal propionic acid and total VFA concentrations were significantly lower in the M and M-LA treatment hens when compared with the NM treatment hens. The number of hens with SE culture-positive crop and ovaries was significantly higher in the M treatment hens than in the NM or M-LA treatment hens (Table 6). The number of hens with SE culture-positive ceca and spleen was significantly higher in the M and M-LA treatment hens than in the NM treatment hens, whereas there were no differences in the number of hens with SE culture-positive livers. The number of *Salmonella* in the crop was significantly higher in the M treatment hens than in the other groups, whereas the M and M-LA treatment hens had significantly higher numbers of *Salmonella* than the NM treatment hens.

DISCUSSION

Given the increasing economic popularity of induced molting in the layer industry, intervention strategies are needed to resist SE colonization and invasion in laying hens during molting. However, intervention strategies must still retain full molt induction capabilities. In the current study, drinking water treatments did not appear to influence the bird molt induction response when compared with feed withdrawal. However, there was a decrease from the original body weight of approximately 25% for hens deprived of feed for 9 d during 3 experiments. When compared with the NM hens without added acetic acid or LA, the quantity of weight lost by the NM

TABLE 4. Effect of treatments on incidence and severity of *Salmonella* Enteritidis (SE) colonization and invasion in experiment 2

	Parameter[1]				
	SE culture-positive hens (%)			Log_{10} *Salmonella*	
Treatment[2]	Crop	Ceca	Liver and spleen	Crop	Ceca
NM	3/12	0/12	8/12	0.61[b]	0[b]
M	7/12	7/12*	8/12	1.48[ab]	2.85[a]
M-LA	10/12*	10/12*	12/12	1.93[a]	4.41[a]
LSD[3]				1.12	2.41

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Values represent the mean of 12 hens per treatment.

[2]NM = full fed-nonmolted; M = feed deprived; M-LA = feed deprived + 0.5% lactic acid in distilled water.

[3]LSD = least significant difference as determined by Fisher's protected LSD procedure.

*Significantly ($P < 0.05$) different from NM control by chi-squared analysis.

TABLE 5. Effect of treatments on body weight changes, ovary weights, crop pH and lactic acid(LA), and cecal propionic acid (PA) and total volatile fatty acids (TVFA) in eperiment 3

	Parameter[1]					
Treatment[2]	BW change (%)	Ovary weight (g)	Crop pH	Crop LA (μmol/crop)	Cecal PA (μmol/g of cecal content)	Cecal TVFA (μmol/g of cecal content)
NM	–2	34.4[a]	4.55[b]	25[a]	19.6[b]	115.7[a]
M	–23	15.3[b]	5.83[a]	3[c]	29.0[a]	83.9[b]
M-LA	–25	13.7[b]	5.64[a]	9[b]	16.6[b]	68.4[b]
LSD[3]		3.52	0.50	3.5	7.43	28.69

[a–c]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Values represent the mean of 12 hens per treatment.

[2]NM = Full fed-non-molted; M = feed deprived; M-LA = feed deprived + 0.5% lactic acid in distilled water.

[3]LSD = Least significant difference as determined by Fisher's protected LSD procedure.

hens receiving acetic acid or LA in the drinking water during the experimental period did not differ. Likewise, ovary weights were decreased comparably in all molted hens.

Inclusion of acetic acid or LA in drinking water did not cause a significant reduction in the number of hens colonized by SE or the numbers of SE in the hens colonized by SE. However, Byrd et al. (2001) conducted experiments with broiler chicks and found a significant increase in resistance to *Salmonella typhimurium* when LA was added to the drinking water of broilers during feed withdrawal. It should be noted that broilers undergo feed withdrawal for several hours, whereas laying hens in this study were deprived of feed for 9 d. This difference in time may account for the difference in response observed between broilers and laying hens. A standard management practice in commercial broiler production is removal of feed immediately prior to transportation to the processing plant. This practice (analogous to feed deprivation in laying hens, except for a shorter period of time) has been shown to increase the number of *Salmonella*- and *Campylobacter*-contaminated crops (Ramirez et al., 1997; Byrd et al., 1998). The normal microflora of the crop have been shown to compete with coliform and pathogenic bacteria. Part of the resident population of bacteria includes *Lactobacilli*, a major producer of LA (Fuller, 1973, 1977; Fuller and Booker, 1974; Humphrey et al., 1993). Feed withdrawal has been reported to increase broiler crop pH and decrease LA concentrations (Humphrey et al., 1993; Corrier et al., 1999b).

A 9-d fasting period may create a substantially different microenvironment, resulting in colonization by SE that is much more difficult to prevent. Longer periods for feed removal from laying hens may be sufficiently traumatic to the indigenous microflora throughout the gastrointestinal tract (crop, intestine, and ceca) that organic acids supplemented in the drinking water are simply too temporal to serve as a barrier to SE colonization. Natural infection of poultry by *Salmonella* occurs via the oral route, and salmonellae colonize the intestinal tract with the crop and ceca being the primary sites of colonization (Brownwell et al., 1970; Soerjadi et al., 1981; Stavric, 1987; Impey and Mead, 1989). Several common themes have emerged on how successful colonization of the chicken intestinal tract by SE, subsequent invasion of organs beyond the intestinal tract, and eventual contamination of eggs occurs (Gast and Beard, 1993; Holt, 1993, 1995, 1999; Thiagarajan et al., 1994). Clearly, feed removal and subsequent emptying of the gastrointestinal tract are critical factors because fasted chickens (Moran, and Bilgili, 1990; Humphrey et al., 1993; Corrier et al., 1999a), mice (Miller and Bohnhoff, 1962), and ruminants (Brownlie and Grau, 1967; Grau et al., 1968) all exhibit increases in populations of *Salmonella* in what would be considered normally hostile gastrointestinal environments. Birds infected with SE during feed withdrawal not only shed significantly higher numbers

TABLE 6. Effect of treatments on incidence and severity of *Salmonella* Enteritidis (SE) colonization and invasion in experiment 3

	Parameter[1]						
	SE culture-positive hens %					Log_{10} *Salmonella*	
Treatment[2]	Crop	Ceca	Liver	Spleen	Ovary	Crop	Ceca
NM	1/12	2/12	2/12	0/12	1/12	0.25[a]	0.57[b]
M	5/12*	8/12*	5/12	5/12*	8/12*	1.49[b]	3.62[a]
M-LA	2/12	10/12*	5/12	4/12*	2/12	0.36[a]	4.55[a]
LSD[3]						1.24	2.13

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Values represent the mean of 12 hens per treatment.

[2]NM = Full fed-nonmolted; M = feed deprived; M-LA = feed deprived + 0.5% lactic acid in distilled water.

[3]LSD = least significant difference as determined by Fisher's protected LSD procedure.

*Significantly ($P < 0.05$) different from NM Control by chi-squared analysis.

SE from the intestinal tract, but the organism is distributed more evenly along the intestinal tract (Holt and Porter, 1992; Holt et al., 1995). More importantly, SE colonization is extremely rapid in these birds. Within 48 h of feed withdrawal, significantly more SE can be recovered from the livers and spleens of molted birds compared with fed nonmolted birds (Holt et al., 1995). This in turn leads to increased horizontal spread of infection to molted hens in neighboring cages (Holt and Porter, 1992, 1993; Holt, 1995; Holt et al., 1998).

It appears that feed removal has little effect on microbial factors that would be considered inhibitory in the cecal microenvironment (lower pH, decreased oxidation potential, and increased VFA concentrations) when feed-deprived and molted birds are compared with fed and nonmolted birds (Holt et al., 1994; Corrier et al., 1997). However, hens and broilers exhibit increased *Salmonella* crop contamination during feed withdrawal (Humphrey et al., 1993; Ramirez et al., 1997; Corrier et al., 1999a). Because *Salmonella* initially encounters the crop environment during oral infection of poultry, the crop environment may be an important determinant of subsequent SE intestinal colonization invasion. Durant et al. (1999) observed that a 9-d feed withdrawal substantially increased SE antibiotic marker strain colonization of the crop (6-fold increase in the number of hens with SE-positive crops) and ceca (3 $\log_{10}$ increase in SE colonization) of molted birds vs. nonmolted birds. In addition, invasion of the spleen and liver of molted birds was increased at least 5-fold compared with nonmolted birds.

Based on the results of the current study, additional research with different acid-water treatments in conjunction with some alternative molt feed regimens may prove to be more effective in reducing the incidence of SE in eggs and internal organs during and after molting of laying hens.

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